Effect of Predisposing Temperatures on The Histopathology of The Rice Blast Fungus, Pyricularia oryzae

I. Effect of Blast Fungus Isolates on Penetration of Rice Varieties at Different Predisposing Temperature Regimes

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ABSTRACT

Percent penetration on a specific rice variety was more affected by blast fungus isolate or predisposition temperature than by temperature and isolate combinations. A susceptible variety tested remained continuously susceptible regardless of whether the variety was grown at different temperature regimes and exposed to isolates/races with differences in pathogenicity and virulence. The expression of virulence by a particular bíast fungus isolate race was observed to be changed by subjecting rice host plants to different predisposing temperature conditions prior to inoculation.

INTRODUCTION

Rice blast, caused by *Pyricularia oryzae*, is of major economic importance among the diseases of rice and has been reported to occur in some sixty countries (Parthasarathy et. al., 1965). Coons (1953) has stated that the control of disease by

use of a disease-resistant variety is a painless method that does not levy on the farmer's pocket-book except as he has to pay for the care and harvest of a larger crop. Recent experiences in Korea with the rice blast epidemic of 1978 have shown the follacy of this statement. Resistant varieties do not necessarily remain resistant indefinitely and for scientists, rice researchers and rice

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producers to avoid calamities such as blast epidemics it is necessary to have a complete and thorough understanding of the disease. As rice production becomes more and more intensive and as the human population continues to increase, the consequences of an epidemic of rice blast become increasingly more serious.

The penetration phase of pathogenesis is one of the deficient areas of knowledge with regard to the rice blast disease, Many factors affecting pathogenesis of the blast fungus are well documented by several researchers (Ito et. al., 1931b; Yousii, 1936; Suzuki, 1940; Suzuki, 1969b; Kato et. al., 1970). However, very little information is available concerning the penetration of the rice plant by the blast fungus, especially on the leaf epidermis (Yoshino, 1972).

The main objective of this research was to study the differences in penetration by pathogenic and non-pathogenic races of the blast fungus on resistant and susceptible rice varieties.

MATERIALS AND METHODS

Test Varieties

The varieties Tetep, IR36, Carreon, Sensho, Khao-tah-haeng 17 (KTH) and Peta were used throughout the course of study. These varieties, as well as the fungus isolates, were selected on the basis of pathogenicity studies conducted by the Plant Pathology Department of the International Rice Research Institute (IRRI). Seeds of each variety were increased by the Plant Pathology Department and the same seed lots were used in all experiments.

Test Isolates

Six isolates, namely: 2017, 4702, L-1441, 750678 -1, 1509 and 2137 were used (Table 1). These isolates were obtained from permanent stock cultures maintained by the Plant Pathology Department, IRRI.

Sowing

Two sets of the six varieties with eight pregerminated seedlings per 10 cm row were sown in

Table 1. General reaction of six isolates of *Pyricularia oryzae* on six rice varieties.

Isolates	Tetep	IR36	Carreon	Sensho	KTH	Peta
2017	Sª	R	R	R	S	 S
4702	R	R	R	S	S	S
750678-1	R	S	R	M	S	
L-1441	R	_	R		S	R
1509	R	_	R		R	S
2137	R	S	R	S	S	S

^aR=No symptoms or hypersensitive; S=typical lesions produced; —=reaction not confirmed.

one plastic tray $(25\times32\times12 \text{ cm})$. Each plastic tray contained 8 kg of soil mixed with 2 g of NH₄ $(SO_4)_2$, 0.4 g of calcium phosphate and 0.4 g of KCl.

After sowing, the plastic trays were placed in separate temperature controlled rooms until time of inoculation. Plants were grown for approximtely 20 days (3~4 leaf stage).

Temperature Conditions for Seedling Growth

Potted seedlings were grown in the Phytotron with controlled temperatures and 70% relative humidity. The different temperature conditions were 29/21C (high daytime temperature from 9:00 a.m. to 5:00 p.m. and low night temperature from 5:00 p.m. to 9:00 a.m.), 32/24C and 35/27 C. Other sets of potted seedlings were grown in the greenhouse where temperature, relative humidity and light intensity were uncontrolled and variable.

Sporulation

While plants were being grown for inoculation, eight-day old prune agar slant cultures were scraped with 10 ml prune extract (Table 7) and equally plated in five prune agar petri dishes. The plates were initially kept for three days in 24C incubator and than transferred to an incubation shelf with near-ultraviolet light where they were allowed to sporulate for 7 days.

Inoculation

Wooden frame boxes $(90 \times 75 \times 140 \text{ cm})$ covered with plastic sheets and cloth linings were used as inoculation chambers (Ahn, 1977). A few hours

prior to inoculation, the inside of the chamber was sprayed with an adequate amount of water to maintain free water on the plants during inoculation. One plastic tray of seedlings which was grown under each of the different temperature conditions was placed in one chamber and inoculated with 50 ml of spore suspension using an electric motor sprayer to insure an even and uniform distribution of spores. The concentration of spores was 1.5~2.0×105 spores/ml. Isolates were inoculated separately in each chamber. The plants were maintained for 24 hours inside the inoculation chamber at a temperature of 25±1C and then were transferred to a seperate greenhouse incubation room with humidifier and fan cooling system for six days. Five new fully expanded leaves of each variety per isolate per temperature regime were collected and fixed in FAA solution 72 hours after inoculation (Yashino, 1972) for microscopic observation and determination of percentages of germination, appressorium formation and penetration.

Histopathological Method

Yoshino's histopathological method (Yoshino, 1971) was used throughout this investigation for the determination of appressorium formation and penetration rates on the leaf blade epidermis. The procedure consisted of the following steps:

Sample fixation: FAA No. 2 solution 72 hours after inoculation

Silica removal: 0.5% HF solution in 50% ethanol for 3 days

Washing: 2~3 times with tap water

KOH treatment: 4% KOH solution for one night and then heat with steam for 30 minutes

Washing: 2~3 times with distilled water

HCl treatment: 30 minutes in 23.6% HCl solution

Staining: Boiling for 60 minutes in staining solution (2:1:1 fixative mixture of 0.05% aniline blue in lactophenol, 0,02% aniline blue solution, and glacial acetic acid)

Determination of Number of Silicified Motor Cells

One leaf per treatment was boiled in liquid phenol for 20 minutes and 10 view fields with low

power magnification ($100\times$) were observed and the number of silicified motor cells counted (Hori, 1963).

Determination of Free Sugar Content

One set each of Tetep and KTH samples from six month old stock preserved in FAA solution was analyzed by the Chemistry Department, IRRI, for free sugar content in terms of glucose as measured on a per-leaf basis using the phenol-sulfuric acid method.

Experimental Design, Data Collection and Analysis

Completely randomized design (CRD) with two blocks was used for the foliar inoculation study. Eight pre-germinated seedlings were sown in a randomized 10 cm row within each block. In the penetration study five randomly collected leaves from one block were microscopically examined.

The specific variables and interactions which were measured and analyzed in this study are listed below.

- 1. Effect of isolate and predisposing temperature on percent conidia germination,
- 2. Effect of isolate and predisposing temperature on appressoria formation,
- Effect of isolate and predisposing temperature on penetration,
- 4. Interaction of isolate by temperature on penetration, and
- 5. Relationship between predisposing temperature and variety within single isolate with respect to penetration.

RESULTS AND DISCUSSION

Predisposition of six rice varieties at four different predisposing temperature regimes had no significant effect on percent germination of conidia and appressorium formation of six different isolates of the blast fungus, *Pyricularia oryzae*. Percent penetration, however, was affected by isolates, varieties and predisposing temperature regimes.

Percent germination of conidia uniformly deposited on the leaf surface ranged from 94 to 100%.

In most cases, regardless of blast isolate, variety and temperature regime, percent germination of conidia was about 99%. Germination rate of conidia was shown to approach 100%, 10 hours after incolation at 25±1C regardless of light and dark treatment. (Kim et. al., 1974). The results by many researchers suggested that the optimum temperature for spore germination was 25C (Nisikado, 1927; Sueda, 1928; Sadasivan et. al., 1965).

Percent appressorium formation ranged from 94 to 100% but mostly reached 98% completion. No significant differences for appressorium formation by isolates, varieties and temperature regimes were detected. There appears to be divergent opinions among researchers on the effect of temperature on appressorium formation. Ito et. al. (1931a) concluded that appressorium formation was better at 15~18C than at 28C even with reduced germination at lower temperatures. Appressoria were formed 15 hours after incubation at 20 ~32.5C and 24 hours after incubation at 25~35C (Suzuki, 1941). The optimum temperature was 28C for certain races (Suzuki, 1952), but different temperatures were required for appressorium formation for different races (Fujikawa et. al., 1954). Suzuki (1969a) reported the optimum temperature was 20C but that appressoria were formed well at 25, 16, and 14C. Yoshino (1973) concluded 24C appeared to be optimum for appressorium formation. Later experiments indicated that appressorium formation began 4 hours after inoculation and reached 60~80% completion 15 hours after inoculation (Kim et. al., 1974). In the present study percant germination of conidia and appressorium formation by isolate I-L-1441 were generally lower on all varieties when they were predisposed at the 32/24C regime.

Differences in percent penetration were detected and varied from zero in Tetep, Carreon, IR36 and Sensho for some isolates and temperature combinations to 5.9% in Khao-tah-haeng 17 (KTH) with isolate I-750678-1 at 29/21C (Table 4a). However, percent penetration in general was considered to be relatively lower than expected, and there are several possible reasons for this. First, is the possible involvement of resistance mechanisms in

the rice varieties acting aganist the blast fungus. isolates. During the microscopic observations, it was frequently observed that the epidermal cells. (mostly motor cells) of the resistant varieties Carreon and Tetep were stained blue by aniline blue without the occurrence of penetration pegs from appressoria. One possible explanation for this phenomenon may be the presence of phytoalexins which are antibiotics produced by the host-parasite interactions or as a response to injury, physiological stimuli or the presence of infectious agents (Ku5, 1975). In accordance with the phytoalexin theory (Wheeler, 1975) it may be assumed that compounds with phytoalexin properties. may have been produced and were accumulated in the rice varieties in response to inoculation with the blast fungus isolates and these compounds were produced in greater amounts in resistant varieties. Thus, cells of the resistant varieties Carreon and Tetep could be expected to be stained blue without the occurrence of infection or penetration.

Secondly, the physiological processes of rice varieties might be changed or influenced by the predisposing temperature treatment. In this study, the mean number of silicified motor cells and the mean free sugar content were measured. In general, there appeared to be a negative relationship between percent penetration and the mean number of silicified motor cells with respect to temperature except for Sensho (Table 2). A negative correlation between number of silicified motor cells and occu-

Table 2. The mean number of silicified motor cells of six rice varieties grown under four different predisfosing temperature regimes.²

Variety	GH	29/21 C	32/24 C	35/27 C
Carreon	80.0	51.1	65.2	146.8
IR36	35.1	36.1	23.3	179.9
КТН	39.1	29.0	45.1	61.0
Peta	98.0	47.1	42.3	85.9
Sensho	2.7	75.8	29.6	7.0
Tetep	43.9	37.7	76.4	119.1

Average of 10 microscopic view field at 100x magnification.

rrance of panicle blast has been documented by Hori (1963) and Kim et. al. (1977) demonstrating the role of silicified motor cells in reducing the incidence of blast. On the other hand, Tetep had a higher mean free sugar content than KTH but there appeared to be no direct relationship among the temperature regimes within varieties for sugar content (Table 3). It is generally accepted that

Table 3. Mean free sugar content (mg glucose /g fresh weight) in KTH and Tetep rice varieties grown under four different predisposing temperature regimes.

Temperature ^b	KTH	Tetep
GH	85.1	150.00
29/21 C	122.22	178.78
32/24 C	122.22	150.00
35/27 C	104.44	121.73

Average of 5 leaves at six months after preservation in FAA No. 2 solution.

rice plants with higher nitrogen content are more susceptible to blast, but with regard to the relationship of sugar content and disease severity contradictory opinions exist among researchers. High sugar content has been reported to reduce the susceptibility of rice plants to blast (Terao, 1934; Kozaka et. al., 1954; Yamaguchi Agr, Exp. Sta., 1961; Ohata et. al., 1966); however, increased susceptibility was reported by Tahara (1937), Samborski et. al. (1960) and IRRI (1975). Over dressing of nitrogen fertilizer was reported to increase total nitrogen and decrease silicate and total sugar content in leaf blades resulting in rice plants which were more susceptible to the blast disease (Paik, 1975). It has been reported that sugar content of rice leaves is quite variable and there is no direct relationship between sugar content and blast susceptibility (Otani, 1959; Tokunaga, 1959). Ohata et. al. (1966) investigated this relationship by using extremely shaded conditions and reported that decreased sugar content in the rice plant ifself became a limiting factor for fungal growth which in turn resulted in reduced susceptibility of rice plants to blast. However, when the sugar content in the rice plants was increased above a certain level, he concluded the reduction of amino acids or increase of phenol compounds accompanied by an increase in sugar content affected growth of the fungus and resistance of rice plant resulting in an eventual decrease in susceptibility (Ohata et. al., 1966). Therefore, it is difficult to conclude that silicified motor cells and glucose content are the direct reasons for the low percent penetration in this study.

Thirdly, the difference in virulence of isolates appeared to play a role in lowering percent penetration. It is interesting to note the relationship of I-2017 and I-1509 for KTH. According to pathogenicity studies conducted by the Plant Pathology Department, IRRI(Table 1), KTH showed a susceptible reaction to I-2017 and a resistant reaction to I-1509. In this study, I-1509 appeared to be more virulent than I-2017 on KTH at all temperature regimes. In an inoculation study by Yoshino (1972), percent penetration in the resistant rice varieties Senshurak and Ishigarishiroge by a pathogenic rice of Pyricularia oryzae designated as N-2 was 0.4 and 12.7%, respectively, 72 hours after inoculation. Percent penetration in this study for all isolates, varieties and predisposing temperatures varied from 0 to 5.9%.

Among the six isolates tested, I-750678-1 was the most virulent as measured by percent penetration on all vaieties (Table 4a). I-750678-1 successfully penetrated all varieties at all temperature conditions. Isolates 750678-1, 2137, 1509, L-1441, 2017 and 4072 successfully penetrated 24, 21, 20, 19, 19 and 18 times, respectively, when each variety at each predisposition temperatures was considered to present one chance of penetration(Table 4a). At 29/21C (day/night temperature considered most optimum for infection to occur) all six blast isolates were able to penetrate all six varieties although penetration in some varieties with some isolates was quite low. Percent penetration at 29 /21C ranged from 0.075% for I-2137 on Sensho to 5.875% for I-750678-1 on KTH.

^{*}GH=Uncontrolled and variable greenhouse conditions; first number refers to 8 hours constant day temperature, second number 16 hours constant night temperature.

Table 4a. Percent penetration of six *Pyricularia oryzae* isolates on six rice varieties under four different predisposing temperature regimes at 72 hours after incoulation and maintenance at 25C temperature.

Predisposing	37		Isolates				
Temperature Regime ^{ab}	Variety	2137	L-1441	2017	4702	1509	750678-1
GH	Carren	0.725 bc ^c	0.497 c	0.295 b	0 b	1.234 cd	0.275 d
	IR36	0.535 c	2.042 ab	0 c	0.468 a	1.179 bc	0.814 c
	KTH	3.210 a	1.001 bc	2.010 a	1.408 a	4.528 a	3.946 a
	Peta	1.669 b	2.475 a	1.280 a	0.789 a	2.008 b	2.286 b
	Sensho	0.558 c	0.091 d	0 c	1.022 a	0.493 cd	1.164 b
	Tetep	0.074 d	0 d	0.997 ab	0 b	0.201 d	0.294 d
29/21 C	Carreon	0.415 cd	0.199 b	0.663 ab	0.268 bc	0.505 b	1.485 b
	IR36	0.582 bc	0.514 b	0.201 b	0.100 c	0.253 b	2.453 b
	KTH	2.112 a	4.120 a	1.215 a	1.461 a	2.701 a	5.875 a
	Peta	1.342 ab	2.061 a	1.168 a	1.137 a	2.122 a	5.251 a
	Sensho	0.075 d	0.196 b	0.210 b	0.676 ab	0.332 b	2.759 b
	Tetep	0.108 d	0.146 b	0.804 ab	0.097 c	0.283 b	0.501 c
32/24 C	Carreon	0.335 b	0.196 b	0.100 c	0.241 bc	0 b	0.159 d
	IR36	0 Ъ	1.583 a	0 c	0.111 bc	0 b	1.232 b
	KTH	1.926 a	3.037 a	0.672 ab	0.587 a	2.081 a	3.658 a
	Peta	1.067 a	2.652 a	0.199 bc	0.412 ab	1.227 a	1.617 b
	Sensho	0 b	0 b	0.101 c	0.095 bc	0.087 b	1.343 b
	Tetep	0.132 b	0 b	1.027 a	0 с	0.099 b	0.613 c
35/27 C	Carreon	0.253 bc	0.097 b	0 b	0.095 a	0.097 b	0.149 c
	IR36	0.183 bc	0.647 a	0 b	0 a	0 b	0.580 b
	KTH	1.311 a	1.370 a	0.190 ab	0.108 a	1.551 a	1.819 a
	Peta	0.324 b	0.746 a	0.312 a	0 a	1.286 a	1.330 a
	Sensho	0.098 bc	0 b	0.097 ab	0.102 a	0 b	0.297 c
	Tetep	0 с	d 0	0.542 a	0 a	0.103 b	0.097 c

[&]quot;The plants were maintained 24 hours inside the inoculation chamber at a temperature of 25±1C and transferred to a separate incubation room.

KTH and Peta, as expected based upon the preliminary studies, exhibited the highest percent penetration in most of the variety by isolate by predisposition temperature combination. The frequency of penetration by all isolates was similar on Tetep and IR36 but percent penetration was much lower on Tetep than IR36 (Table 4b).

KTH and Peta were penetrated at a significantly higher rate than the other varieties (Table 5).

These interactions indicated that a susceptible variety remained continuously susceptible even when the variety was grown at different temperature regimes and exposed to isolates/races with differences in pathogenicity and virulence.

I-750678-1 was the most virulent of all six isolates at 29/21C on all varieties (Table 4b). The percent penetration of I-750678-1 at other temperature regimes was less than at 29/21C. The ability

^bGH=refers to greenhouse where temperatures were variable and fluctuated from 33 to 23C. The first number refers to a constant day temperature and the second to a constant night temperature, each 8 and 16 hours in duration.

^{&#}x27;In a column, means followed by a common letter are not significantly different at the .05 level by DMRT.

Table 4b. Percent penetration of six P. oryzae isolates under four different predisposing temperature regimes at 72 hours after inoculation and maintenance at 25°C temperature.^a

Predisposing			Varieties				
Temperatare Regime ^b	Isolate	Carreon	IR36	KTH	PETA	Sensho	Tetep
GH	2137	0.725 a°	0.535 a	3.210 ab	1.660 a	0.558 ab	0.074 b
	L-1441	0.497 a	2.042 a	1.001 c	2.475 a	0.091 bc	0 b
	2017	0.295 ab	0 b	2.010 bc	1.280 ab	0 c	0.997 a
	4702	0 b	0.468 a	1.408 c	0.789 b	1.022 a	0 b
	1509	1.234 a	1.179 a	4.528 a	2.008 a	0.493 ab	0.201 b
	750678-1	0.275 ab	0.814 a	3.946 ab	2.286 a	1.164 a	0.294 b
29/21 C	2137	0.415 ab	0.582 b	2.112 c	1.342 b	0.075 b	0.108 a
	L-1441	0.199 b	0.514 b	4.120 ab	2.061 b	0.196 b	0.146 a
	2017	0.663 ab	0.201 b	1.215 c	1.168 b	0.210 b	0.804 a
	4702	0.268 b	0.100 b	1.461 c	1.137 b	0.676 b	0.097 a
	1509	0.505 ab	0.253 b	2.701 bc	2.122 b	0.332 b	0.283 a
	750678-1	1.485 a	2.453 a	5.875 a	5.251 a	2.759 a	0.501 a
32/24 C	2137	0.335 a	0 b	1.926 ab	1.067 bcd	0 b	0.132 bc
	L-1441	0.196 a	1.583 a	3.037 a	2.652 a	0 b	0 c
	2017	0.100 a	0 b	0.672 bc	0.199 d	0.101 b	1.027 a
	4702	0.241 a	0.111 b	0.587 bc	0.412 cd	0.095 b	0 c
	1509	0 a	0 b	2.081 a	1.227 abc	0.087 b	0.099 bc
	750678-1	0.159 a	1.232 a	3.658 a	1.617 ab	1.343 a	0.613 ab
35/27 C	2137	0.253 a	0.183 ab	1.311 a	0.324 ab	0.098 a	0 b
	L-1441	0.097 a	0.647 a	1.370 a	0.746 a	0 a	0 b
	2017	0 a	0 b	0.190 b	0.312 ab	0.097 a	0.542 a
	4702	0.095 a	0 b	0.108 b	0 b	0.102 a	0 b
	1509	0.097 a	0 b	1.551 a	1.286 a	0 a	0.130 ab
	750678-1	0.149 a	0.580 a	1.819 a	1.330 a	0.297 a	0.097 ab

^aThe plants were maintained 24 hours inside the inoculation chamber at a temperature of 25±1C and transferred to a separate incubation room.

of the various isolates to penetrate the different host varieties grown at different temperature conditions was variable and suggested that the expression of virulence by a particular isolate/race may be changed by exposing the host to different predisposing temperature conditions (Table 4b).

Significant differences among isolates, predisposing temperature regimes and varieties for penetration were observed (Table 6). The interactions between predisposition temperature by variety as well as isolate by variety were highly significant while the temperature by isolate by variety interaction was only significant.

Percent penetration on a specific variety was more affected by isolate or predisposition temperature than by temperatures and isolates combined (Table 6). For example, Tetep was not penetrated by isolates L-1441 and 4702 at any predisposing temperature regime except 29/21C (Table 4b). Likewise, IR36 was penetrated by I-2017 at 29/21C

^bGH=refers to greenhouse where temperatures were variable and fluctuated from 33 to 23C. The first number refers to a constant day temperature and the second to a constant night temperature, each 8 and 16 hours in duration.

^{&#}x27;In a column, means followed by a common letter are not significantly different at the .05 level by DMRT.

Table 5. Interaction between four predisposing temperature regimes and six rice varieties for percent penetration.

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Variety	Correlation coefficient
КТН	682**a
Peta	678**a
Carreon	437**ab
Sensho	388**ab
IR36	340**ab
Tetep	~. 210*b

^{***:} Significantly different at 1% level.

Table 6. Analysis of variance for percent penetration of six blast isolates on six rice varieties at four predisposing temperature regimes at 72 hours after inoculation.*

SV	DF	MS	F-Value
Replication	1	101.9723	
Isolate	5	87.8437	11.09***
Error (A)	5	7.9192	
Temperature	3	113.6694	20.00**
Isolate×Temperature	15	8.6979	1.53"
Error (B)	18	5.6829	
Variety	5	283.8836	147.48**
Isolate ×Variety	25	20.7805	10.80**
Temperature×Variety	15	4.4569	2.32**
Isolate × Temperature × Variety	75	3.0029	1.56*
Error (C)	120	1.9245	
Total	287		

CV(A) = 70.5%

but not at the other three predisposing temperature regimes. Furthermore, I-2137 did not penetrate IR36 and Sensho at 32/24C or Tetep at 35/27C but penetration occurred at all other predisposing temperature regimes. I-1509 did not penetrate IR36 at predisposition temperatures of 32/24C or 35/27 C but did penetrate at the other two predisposing

Table 7. Composition of prune agar.

Amount		
11		
5g		
1g		
20g		

^{*}One prune extracted with 11 of water.

temperature regimes. Carreon was not penetrated by I-1509 only at the 32/24C predisposing temperature regime.

摘 要

特定水稻品種에 對한 稻熱病菌의 侵入率은 接種前處 理溫度와 菌株의 組合보다는 供試菌株나 溫度의 單獨 要因에 依한 影響이 컸다. 罹病性品種은 그 品種이 어 면 溫度條件下에서 자랐던지 또는 病原性이 다른 어떤 菌株로 接種이 되었던지 罹病性으로 남아 있었으며 接 種前에 寄主가 다른 溫度條件下에서 자랐을 境遇, 稻. 熱病菌의 特定菌株 또는 Race의 病原性發現은 다르게 나타나는 것이 觀察되었다.

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^{*:} Significantly different at 5% level.

CV(B) = 59.7%

CV(C) = 34.8%

[&]quot;Analysis is based on values transformed to squre

^{***:} Significantly different at 1% level.

^{*:} Significantly different at 5% level.

ns: Not significantly different.

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